

## **Effects of Combination Ethephon and Urea on Productivity of *Jatropha curcas* L. Thai Accession**

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### **ABSTRACT**

Low number of fruit production becomes the main constraint to develop *Jatropha curcas* as one of the alternative biodiesel sources in Malaysia. Therefore, one study was carried out to evaluate the effects of ethephon by combining it with urea for induction of flowering in *Jatropha*, with the main objective of increasing the productivity of fruit. The experiment was carried out at Rubber Research Institute Experimental Station (RRIES) Sungai Buloh, Selangor, where the soil was identified as the Renggam series. The planting material used was *Jatropha curcas* from Thailand accession. There were a total of nine treatments which were arranged according to Randomized Complete Design (RCD). Results indicated that Treatment 2 (200ppm ethephon 2.5% + 2% urea) showed the best combination promote in balancing male-female ratio, and resulted in an improved yield of the plants expressed as an increase in fruit produce and ripe fruit. The efficacy of ethephon at a lower concentration for increased fruit yield, with the addition of urea, is of great deal as this will lead to decreased costs and increased survival of *Jatropha* plantation.

*Keywords:* Ethephon, *Jatropha curcas* L., Thailand accession, urea

### **INTRODUCTION**

The price of crude oil was around USD95-100 due to limited production sources and increases in the demand for fuel, and the

price could up above USD100 in certain time ([www.oil-price.net/27 Jun 2013](http://www.oil-price.net/27)). In many countries including Malaysia, biofuel is now an alternative for renewable energy, after wind energy and solar energy. As a consequence, there is high a demand for biofuel materials. Palm, sunflower, rapeseed and *Jatropha* oils are the materials available as biofuel feedstock ([wikipedia.org/wiki/Biodiesel/27 Jun 2013](http://wikipedia.org/wiki/Biodiesel/27)). Biofuel based on

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*Jatropha* requires further investment as supported by a broad body of information from various studies. As *Jatropha* is considered a new and developing crop to the plant community, there has been a great deal of research conducted on it. Based on its potential as an alternative source of biodiesel, the Malaysian government has planned to develop *Jatropha* as one of the commodity crops. The Ministry of Plantation Industries and Commodities through the Malaysian Rubber Board has been appointed as the leader in planning and executing a *Jatropha* project, in terms of its suitability and also the economic impacts.

Meanwhile, oil of *Jatropha* can be extracted from the fruit. However, poor number of fruit has been the major limiting factor for commercialisation of this crop, which has been highlighted in various publications (see Heller, 1996; Biswas *et al.*, 2006; ERIA, 2010.). Pollination of *Jatropha* is one of the limited factors on producing the fruit. It is due to the ratio of the male to female flowers, which is up to 27:1. In addition, Raju and Ezradanam (2002) reported that one of the most reasons for the poor yield is that *Jatropha* has few female flowers resulting from a very low female-to-male flower ratio, which, depending on the genotype, is about 1:29–1:13. Thus, pollination to produce fruit will be difficult due to limited female flowers. According to Bhattacharya *et al.* (2005), only 50% of flowers were reported to fruit due to the inconsistency inflorescence of flowers. One of the solutions is to involve a booster treatment or a growth regulator so as to

improve the number of *Jatropha* fruit (Ghosh *et al.*, 2010; Joshi *et al.*, 2011; Xiurong *et al.*, 2012). However, several researchers have focused on some specific chemicals as the growth regulator, where the high cost involved in the use of these chemical could become the limitation.

Ethephon was widely used in agriculture sector to accelerate pollination process and fruit maturity like application on pineapples (Dass *et al.*, 1975). Unsaturated hydrocarbons like acetylene and ethylene were applied in inducing flowering in pineapple (Lewcock, 1937). However, ethephon (2-chloroethylphosphonic acid) was made available for commercial use. When applied to the plants, the chemical releases ethylene directly in the tissues, producing numerous physiological effects. Ethephon is stable at a pH below 4.0 and since the pH of cytoplasm of plant cells is higher than this, the ethephon is degraded to ethylene as it enters the plant tissues. Generally, urea is known as a supplier of nitrogen (N). Nitrogen is one of the important nutrients in the plant as a component to develop cell tissues and generate photosynthesis activity in plants.

Meanwhile, high concentrations of ethylene (300ppm to 500ppm) can cause dry and necrosis or red colouration in *Ptilotus nobilis* plants in *in vitro* experiment (Prameswara *et al.*, 2009). The same researcher also suggested that high concentrations of ethylene may become toxic to plants. High concentrations of ethylene have been reported to lead to some effects of plant morpho-physiology of *Jatropha*

*curcas*, where the leaf discolouration resulted in the symptom of leave dry (Rosatikah *et al.*, 2012). Therefore, using low concentration of ethylene can reduce the dryness but other added compounds need to be put in so as to maintain the efficiency of ethylene. According to Dass *et al.* (1975), some compounds such as urea and calcium carbonate are able to induce more flowering to pineapples. Therefore, a study was carried out to evaluate the efficacy of different concentrations of ethephon, specifically low concentration, combining it with urea on the productivity of *Jatropha* tree.

## MATERIALS AND METHODS

### *Study Sites*

The study was conducted at Field 15, Rubber Research Institute Experimental Station Sungai Buloh in Selangor, where the soil was identified as the Renggam series. During the experiment, the temperature was around 31-34°C, while the average of humidity was 68%. The planting material used was *Jatropha curcas* from Thailand accession (Fig.1). The age of the *Jatropha* tree during the experiment was 5 years. Latex stimulant (Ethephon 2.5% and Ethephon 5%) was used as the main ingredient in the treatment. Urea used to be urea 46%. The treatments were stated as follows: T1: without spray (Control), T2: 200ppm ethephon 2.5% + 2% urea, T3: 200ppm ethephon 2.5% + 4% urea, T4: 400ppm ethephon 2.5% + 2% urea , T5: 400ppm ethephon 2.5% +

4% urea, T6: 200ppm ethephon 5% + 2% urea, T7: 200ppm ethephon 5% + 4% urea, T8: 400ppm ethephon 5% + 2% urea, T9: 400ppm ethephon 5% + 4% urea.

The treatments were applied as a spray in the month of March to May. The application of the treatments was done once a week. The experimental design was based on a randomized completed design (RCD).

### *Number of Flowers and Fruits*

The number of flowers (male and female) (see Fig.2) was observed and recorded every week. Furthermore, the total fruit per tree was also recorded every week. The ripe fruit was harvested after the colour of fruit had changed from green to yellow (Fig.3). The harvesting period was stopped after one week from the last treatment sprayed. Chlorophyll content was measured using Konica Minolta SPAD Chlorophyll Meter. Meanwhile, the height of the trees was recorded to monitor their growth during the experiment.



Fig.1: Experimental trees of *Jatropha curcas*



Fig.2: The flowers structure of *Jatropha curcas*



Unmatured fruit

Ripe fruit

Fig.3: A comparison between *Jatropha curcas*'s fruit

### Statistical Analysis

The data obtained from the experiment were analyzed by using the Analysis of Variance (ANOVA). The standard error (SE) was evaluated at 5% level of significance. All the experiments were carried in three replicates for each treatment.

## RESULTS AND DISCUSSION

### Number of Fruit Produced

Total fruit in the whole trees for every treatment was determined every week. The results showed that the plants treated with the lowest concentration of ethephon, T2 (200ppm ethephon 2.5% + 2% urea) yielded the highest significance ( $p < 0.05$ ) in the average yield produced during the

experiment (27 total fruits), followed by T3 (15 fruits), T1 (15 fruit), T4 (13 fruits), T9 (12 fruits), T7 (6 fruits), T5 (6 fruits), T6 (5 fruits) and T8 (4 fruits) (Table 1). Meanwhile, during week 0 to week 7, the highest fruit produced was shown for the T2 treatment group with a total of 63 fruits, followed by T3 (31 total fruit), T4 (26 total fruit), T1 (26 total fruit), T9 (22 total fruit), T7 (20 total fruit), T5 (16 total fruit), T6 (15 total fruit) and T8 (14 total fruit). However, from week 8 to week 9, the treatments mostly showed a decreasing trend in the total number of fruit, and this could be attributed to the harvesting period of the ripe fruit.

A considerably higher number of fruit yield was observed in the lowest concentration of 200ppm (i.e., for T2 and T3) added with 2-4% urea compared to the control after the application of the treatment. The high concentrations of ethephon at T5, T6, and T8 did not yield any encouraging results. This showed that the addition of urea had promoted absorption of ethephon and thus increased the availability of ethylene to the plant tissues (Yamada *et al.*, 1965a, 1965b).

The variability in fruit production is generally affected by various factors. In this study, the weather conditions were implied to have influenced flowering. The weather could affect pollination, therefore, stigma receptivity, ovule fertility, ovule longevity and fruit set are directly affected as well (Burgos *et al.*, 1993). In addition, genotype dependent factors related to floral biology influenced fruit set and productivity such as flower bud production and flowering time.

TABLE 1  
The effects of plant growth regulators on the total fruit produce pattern of *Jatropha curcas*

Treatment	Week	Week	Week	Week	Week	Week	Week	Week	Week	Week	Week	Average fruit
	0	1	2	3	4	5	6	7	8	9		
T1 (Control)	Na	Na	1±0.67	1±0.58	4±2.08	5±2.08	33±11.67	26±7	37±8.76	37±4.26	15±4.75 <sup>a</sup>	
T2	Na	Na	1±2.91	13±2.60	20±4.80	52±14.70	52±14.70	63±4.30	52±3.28	11±4.91	27±7.74 <sup>b</sup>	
T3	Na	Na	Na	17±15.52	23±21.50	23±21.5	23±2.93	31±28.3	27±19.6	7±5.43	15±3.87 <sup>a</sup>	
T4	Na	Na	2±1.16	10±2.96	11±3.75	28±1.70	32±3.21	26±6.90	11±1.86	4±2.40	13±3.81 <sup>a</sup>	
T5	Na	Na	Na	3±2.39	9±3.21	9±3.21	9±3.21	16±6.24	4±1.73	18±6.24	6±1.65 <sup>a</sup>	
T6	Na	Na	Na	2±1.15	3±1.33	8±3.67	8±3.67	15±3.53	10±2.30	1±0.33	5±1.68 <sup>a</sup>	
T7	Na	Na	2±0.67	2±1.67	9±8.17	10±8.41	10±8.41	20±15.60	10±7.21	Na	6±2.13 <sup>a</sup>	
T8	Na	Na	1±0.57	3±1.53	3±0.88	3±1.45	4±1.53	14±6.93	12±5.85	Na	4±1.56 <sup>a</sup>	
T9	Na	Na	7±4.37	11±1.53	10±3.84	18±8.09	20±10.84	22±8.89	22±7.02	14±1.45	12±2.45 <sup>a</sup>	

Data are expressed as means ± S.E.

Na: Not available

Mean values with same superscript in column are not significantly different ( $p>0.05$ )

### Number of Ripe Fruit

The results indicated that the plants treated with a lower concentration of mixture ethephon with urea could increase the yield of harvest fruit compared with the untreated plants. The highest results shown for T2 with the concentration of 200ppm ethephon 2.5%, added with 2% urea (Table 2). At the end of the harvesting period, T2 gave the significantly highest number of ripe fruit, with the mean of  $44.33 \pm 2.85$ , followed by T3 with  $30.00 \pm 29.01$ , T1 with  $23.33 \pm 8.99$ , T9 with  $14.00 \pm 11.59$ , T4 with  $13.67 \pm 3.76$ , T5 with  $10.67 \pm 3.84$ , T6 with  $7.33 \pm 4.33$ , and then T7 with  $6.67 \pm 5.70$  and T8 with  $6.00 \pm 2.52$ . However, certain treatments gave some inconvenient results with reference to error bars (i.e., T3 and T9). It could be due to non-uniformed ripe fruit during the experiment. The results of this study are parallel with previous studies which suggested that the ripening of *Jatropha* fruits on the tree is not uniform (Heller, 1996; Biswas *et al.*, 2006). Therefore, in this study, the situation could affect the statistical analysis for ripe fruit, but in average, T2 showed a more pronounced effect on the ripe fruit of *Jatropha*.

TABLE 2

The effects of plant growth regulators on the ripening fruit pattern of *Jatropha curcas*

Treatment	Number of ripe fruit
T1 (Control)	$23.33 \pm 8.99^a$
T2	$44.33 \pm 2.85^c$
T3	$30.00 \pm 29.01^a$
T4	$13.67 \pm 3.76^a$
T5	$10.67 \pm 3.84^{ab}$
T6	$7.33 \pm 4.33^{ab}$
T7	$6.67 \pm 5.70^{ab}$
T8	$6.00 \pm 2.52^{ab}$
T9	$14.00 \pm 11.59^a$

Data are expressed as means  $\pm$  S.E.

Mean values with the same superscript in column are not significantly different ( $p > 0.05$ )

### Number of Flowers

Ethephon treatment can increase the number of inflorescence per plant and balance of both male and female flowers, and thus, increase the trend towards femaleness in comparison to the control. The trend gave encouraging results when treated with T2 (200ppm ethephon 2.5% added with 2% urea), but there was no significant difference ( $p > 0.05$ ) found among the treatment in the flower ratio during the experiment (Table 3). However, T2 had a more pronounced effect on increasing the trend towards femaleness in comparison to other treatment with the average ratio of male to female flowers shown to be  $10.3 \pm 1.90$  during the experiment. Although low concentrations of ethephon were used, the combination with 2% urea could increase absorption into the plant system, and thus, increase the number of inflorescence per plant.



TABLE 3  
The effects of plant growth regulators on the flowering pattern of *Jatropha curcas*

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Average ratio
T1 (Control)	6±1.27	3±1.76	20±1.67	25±3.84	9±1.86	20±5.13	10±2.50	12±2.31	11±4.30	20±2.52	13.6±2.30 <sup>a</sup>
T2	3±0.61	2±0.25	14±0.67	5±1.20	5±1.20	10±2.19	13±2.85	10±2.52	11±4.49	14±0.88	10.3±1.90 <sup>a</sup>
T3	3±1.45	3±0	10±1.45	16±8.09	13±3.76	15±1.45	19±2.30	8±2.03	14±2.31	14±5.36	12.1±1.80 <sup>a</sup>
T4	4±0.64	3±0	16±2.31	9±1.20	4±0.86	31±5.56	32±2.19	10±1.86	12±0.88	30±5.86	14.9±3.70 <sup>a</sup>
T5	3±0.75	3±0.22	21±1.16	19±1.76	7±1.76	17±5.23	15±4.30	24±0.58	14±0.67	20±4.33	14.1±2.38 <sup>a</sup>
T6	2±0.33	1±0.67	17±1.53	24±1.53	6±2.52	21±7.55	16±1.67	17±5.51	22±7.42	20±4.10	14.7±2.70 <sup>a</sup>
T7	3±0.58	2±0.74	19±2.73	24±4.0	9±2.0	32±17.09	11±7.30	24±10.40	8±4.33	18±4.84	14.9±3.20 <sup>a</sup>
T8	3±0.28	2±0.29	12±1.0	27±1.20	19±1.76	27±0.88	21±7.67	12±6.39	21±3.18	12±6.69	15.7±2.82 <sup>a</sup>
T9	2±0.40	2±0.92	13±1.86	12±0.89	12±4.0	28±3.10	12±1.45	12±0.88	19±4.91	30±3.38	14.3±2.90 <sup>a</sup>

Data are expressed as means ± S.E.

Mean values with same superscript in column are not significantly different ( $p>0.05$ )

Inconsistent flower ratio during the experiment could be due to weather conditions, where the number of flowers was affected by water level. Mwanamwenge *et al.* (1999) reported a significant abortion on faba bean flowers and small pods due to water deficit. Guitián (1993) mentioned that drop of flowers is a normal process in many species. It is due to improved fruit set (Jackson & Hamer, 1980). Abdelgadire *et al.* (2008) reported the fruit from cross-pollinated flowers in the crop was significantly larger and numerous than those produced by autogamous self-pollinated flowers.

#### *Chlorophyll Content*

The sprays of ethephon and urea on *Jatropha* plants positively affected the chlorophyll capacity. All the treatments gave increased chlorophyll capacity to the plants after the experiment (Table 4). The highest increment of chlorophyll content was from the plants treated with T8 (400ppm ethephon 5% + 2% urea), with  $33.11 \pm 0.90$  SPAD units to  $38.37 \pm 1.90$  SPAD units, respectively. However, there was no significant difference ( $p > 0.05$ ) among the treatments in terms of the chlorophyll content during the experiment.

TABLE 4  
The effects of plant growth regulators on the biochemical pattern (chlorophyll content) of *Jatropha curcas*

Treatment	Chlorophyll content	
	Before experiment	After experiment
T1 (Control)	$32.40 \pm 1.20^a$	$34.80 \pm 1.21^a$
T2	$33.30 \pm 1.00^a$	$35.87 \pm 2.02^a$
T3	$33.40 \pm 1.20^a$	$35.70 \pm 0.93^a$
T4	$32.20 \pm 0.15^a$	$35.53 \pm 0.19^a$
T5	$32.05 \pm 1.20^a$	$35.53 \pm 1.59^a$
T6	$34.10 \pm 1.10^a$	$37.47 \pm 0.98^a$
T7	$33.60 \pm 1.50^a$	$37.40 \pm 1.46^a$
T8	$33.11 \pm 0.90^a$	$38.37 \pm 1.90^a$
T9	$33.00 \pm 1.20^a$	$36.70 \pm 0.55^a$

Data are expressed as means  $\pm$  S.E.

Mean values with same superscript in column are not significantly different ( $p > 0.05$ )

#### *Height of Trees*

Before the experiment was started, the trees were surveyed and selected to get the average same height of the trees. There was no significant difference ( $p > 0.05$ ) among the heights of the tree in all the treatments (Fig.4) at the start of the experiment. Meanwhile, Fig.5 shows that there is no difference between the heights, and the trend is similar in every treatment during the experiment. These show that the spray of combination ethephon and urea to *Jatropha* trees did not cause any side effect on the growth of the trees and this is in agreement to the findings of Joshi *et al.* (2011).



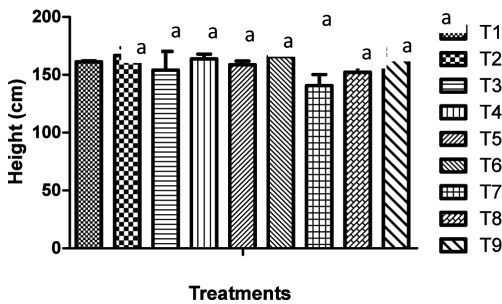


Fig.4: The height of the *Jatropha curcas* trees before the start of the experiment

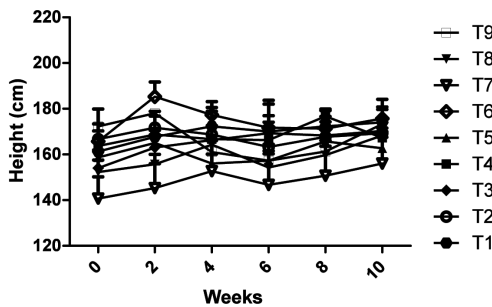


Fig.5: The effects of plant growth regulators on the growth pattern (height) of *Jatropha curcas*

It has been reported that high concentrations of ethylene (300ppm and 500ppm) caused dry and necrosis or red colouration in *Ptilotus nobilis* plants in *in vitro* experiment (Prameswara *et al.*, 2009). In addition, Rosatikah *et al.* (2012) also reported that the application of high ethephon (300 to 600ppm) had induced leaf discolouration to *Jatropha* leaf resulting in the symptom of dry leaves. Thus, the study attempted to develop new mechanisms to use ethephon in low concentrations. Meanwhile, Dass *et al.* (1976) stated that the lowest effective concentration of ethephon which would induce flowering was

previously believed to be around 100ppm. The efficacy of ethephon in increased yield at a low concentration can be improved by the addition of urea. It is probably due to the fact that urea promotes the absorption of ethephon and thus increases the availability of ethylene to the plant tissues (Yamada *et al.*, 1965a, 1965b). In the present study, it was that ethephon added with urea could cause variable effects on different physiological properties of the *Jatropha* plants. The present results indicated that the concentration for pronounced effect when compared with the control, with the concentration of 200ppm (ethephon 2.5%) which was added with 2% urea.

The results presented in Table 1 and Table 2 show that the plants sprayed with low concentration of ethephon added with 2% urea had a significant increase in the fruit produced and ripe fruit. According to Joshi *et al.* (2011), this phenomenon could be attributed to the balanced water and also the concentration of ethephon use during vegetative phase, where stress condition was reduced during reproductive phase and there was an increase in the number of fertile female flowers. In addition, urea could promote increased photosynthesis activity to produce fruit.

The results shown in Table 3 indicate that the combination of ethephon and urea increased the number of inflorescence and balanced the flower ratio compared to the control, especially T2. The treatments with spray on the plants released ethylene and then increased the ethylene concentration at the meristem that might have induced the

development of floral organs. In addition, the efficacy of ethephon in inducing flowering by the addition of urea, even at a low concentration, was probably due to the fact that urea had promoted absorption of ethephon and thus increased the availability of ethylene to the plant tissues (Yamada *et al.*, 1965a, 1965b). Nitrogen also gives response to the metabolic demands of developing reproductive and vegetative organs (Klein & Weinbaum, 1984; Fernández-Escobar *et al.*, 2004). A lesser amount nitrogen will affect fruit set, yield and shoot growth (Freeman *et al.*, 2005). In addition, nitrogen fertilization increased fruit set in rain-fed olives (Hartmann, 1958; Cimato *et al.*, 1990). Meanwhile, Therios (2006) stated that nitrogen increased the proportion of hermaphrodite flowers and a concentration of less than 1% in the leaves led to the formation of staminate flowers and therefore decreased the potential level of fruit set.

Joshi *et al.* (2011) suggested that a suitable ethylene concentration might have increased the growth rate leading to a large apical meristem and greater number of inflorescence. The balance between the male and female flowers per plant in treating plants and the increase in the number of inflorescence in treating plants might be due to the synergistic effects of ethylene on the concentration of other hormones such as gibberellins and cytokinin within the cell (Joshi *et al.*, 2011). The increase in femaleness after ethephon applications may be also related to effect of ethylene on auxin and gibberellins (GA) concentrations *in vivo* and their interaction (Joshi *et al.*, 2011).

It is important to highlight that the chlorophyll content of *Jatropha* plants was increased after the application of ethephon and urea (Table 4). The effects of ethephon and urea on chlorophyll content might be due to the broadening of exterior mesophyll cells that provided more spaces for chloroplast arrangement (Joshi *et al.*, 2011). Meanwhile, Fleischer (1934) reported that the rate of photosynthesis is proportional to the content of chlorophyll. The increase in the chlorophyll content for treating plants in the experiment could contribute to the increase in the number of inflorescence flowers and fruit.

## CONCLUSION

In conclusion, the application of ethephon added with urea has been found to be beneficial for plant development and increasing the yield of the *Jatropha curcas* plants, although at low concentrations. It is suggested that 200ppm ethephon 2.5% added with 2% urea promotes balanced male-female ratio, and thus results in improved yield of the plants expressed as an increase in fruit production and fruit ripening.

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